

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY


(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 04 MAY 2005

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Applicant's or agent's file reference P0121-096/04-JA	FOR FURTHER ACTION		See Form PCT/PEA/416
International application No. PCT/EP2004/003219	International filing date (day/month/year) 25.03.2004	Priority date (day/month/year) 26.03.2003	
International Patent Classification (IPC) or national classification and IPC C12Q1/68			
Applicant PROGENIKA BIOPHARMA, S.A. et al.			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau) a total of 3 sheets, as follows:</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand 26.01.2005		Date of completion of this report 03.05.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized Officer Bradbrook, D Telephone No. +49 89 2399-7413	



**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/EP2004/003219

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-26 as originally filed

Sequence listings part of the description, Pages

1-5 as originally filed

Claims, Numbers

1-20 received on 28.01.2005 with letter of 26.01.2005

Drawings, Sheets

1/5-5/5 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-20
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	1-20
Industrial applicability (IA)	Yes: Claims	1-20
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

**INTERNATIONAL PRELIMINARY REPORT
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Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment on .
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional observations, if necessary:

Section V

1. Reference is made to the following documents:

D1: Cappellen et al., Nature Genetics, Vol.23, p.18-20 (1999)
D2: WO0068424

- 2 The applicant's observations submitted with the amended claims have been considered in establishing this report.

3. Novelty and Inventive step (Art.33(2) and (3) PCT)

- 3.1 None of the prior art documents discloses the subject-matter of claims 1-20 which are therefore considered to be novel (Art.33(2) PCT)

- 3.2 D1 discloses that activating mutations in the FGFR3 gene are detected in bladder carcinomas (cf Table 1), and that in all samples with mutated FGFR3, the mRNA levels of the FGFR3b variant (the only form expressed in both malignant and non-malignant epithelial tissue) were similar to or higher than those encountered in normal bladder epithelium (p.19, col.2, l.2-5).

- 3.3 Claim 1 differs from D1 in that it specifically relates to bladder transitional cell carcinoma (TCC). Furthermore, D1 does not disclose an actual method for diagnosis or prognosis of TCC but merely points out said relationship (cf supra).

- 3.4 Knowing from D1 that FGFR3b expression increases in some instances of bladder carcinoma, it would be obvious and routine for the skilled person to establish the significance of FGFR3b expression as a marker of TCC, which is the commonest type of bladder cancer (cf present description, p.1, l.23-25) and to base a diagnostic test on such expression analysis, thereby arriving at the subject-matter of claim 1.

- 3.5 It should be noted that by referring to the FGFR3b mRNA levels as being "similar to or higher than" those in normal bladder, D1 does not teach away from the method of claim 1. It would not be expected that increased expression is seen in all tumour

samples, and D1 does not disclose exactly what proportion of tumour samples showed increased expression. Nevertheless, the mere teaching from D1 that increased expression is seen in some samples would be sufficient for the skilled person to investigate relative expression levels of FGFR3b as a marker.

- 3.6 Therefore, the subject-matter of claim 1 does not involve an inventive step, contrary to the requirements of Art.33(3) PCT.
- 3.7 Similarly, in the light of D1, it would be routine for the skilled person to screen for suitable therapeutic compounds by applying candidate compounds to cultured bladder tumour cells and determining the effect on FGFR3 expression levels (see also D2: p.7, l.11-21). Therefore, the subject-matter of claim 17 is not inventive (Art.33(3) PCT).
- 3.8 Dependent claims 2-16 do not appear to contain any additional features which, in combination with the features of the claims to which they refer, would render them inventive in the sense of Article 33(3) PCT. Thus, claims 2-13, 15 and 16 relate to routine variations which the skilled person would choose without any inventive activity. With respect to claim 14, the design of primers for amplification of a known sequence is not considered to involve inventive activity, unless the primer pair is shown to have some unexpected property.
- 3.9 D2 discloses primers for amplifying portions of the FGFR3 gene (Example 4), and also refers to kits comprising appropriate reagents (p.35-36). The design of further primers for amplification FGFR3 and their packaging into a kit is not considered to involve inventive activity, unless the primer pair is shown to have some unexpected property. This has not been shown in the present case. Therefore, the subject-matter of claims 18-20 does not involve an inventive step (Art.33(3) PCT).
4. Further comments
- 4.1 Claim 1 is unclear and not supported by the description (Art.6 PCT). Said claim is directed to a method comprising the detection and quantification of the FGFR3 protein or mRNA in a sample from an individual and comparison of the amount

thereof with a normal reference value. However, the term "normal reference values" has no recognised meaning in the art, so that it is unclear with what value the expression level should be compared. Thus, an essential feature, namely the nature of the reference measurement is missing from the claim (cf also p.5, l.12).

- 4.2 The expressions "preferably" and "such as", used in claims 4, 11 and 15 have no limiting effect on the scope of a claim: thus, any feature following any such expression is regarded as entirely optional (cf PCT Guidelines §5.40).
- 4.3 Claim 20 defines merely a particular intended use for the kit of claim 19 and as such is superfluous (Art.6 PCT).

CLAIMS

1. An *in vitro* method to detect the presence of bladder transitional cell carcinoma in an individual, to determine the stage or severity of said cancer in an individual or to monitor the effect of the therapy administered to the individual with said cancer, that comprises
 - a) the detection and quantification of the FGFR3 protein or the mRNA of the *FGFR3* gene in a sample of an individual, and
 - b) the comparison of the amount of FGFR3 protein or the mRNA in said sample with their normal reference values.
2. Method according to claim 1 in which the sample to be analysed is a sample of bladder tissue.
3. Method according to claim 2 in which the sample of bladder tissue is obtained by any conventional method, preferably by cystoscopy.
4. Method according to claim 1 in which the sample to be analysed is a sample of urine, blood, plasma, pleural fluid, ascitic fluid, synovial fluid, bile, semen or cerebrospinal fluid.
5. Method according to claim 1 in which the sample to be analysed is obtained from an individual not previously diagnosed with bladder transitional cell carcinoma.
6. Method according to claim 1 in which the sample to be analysed is obtained from an individual who has been previously diagnosed with bladder transitional cell carcinoma.
7. Method according to claim 1 in which the sample to be analysed is obtained from an individual receiving treatment, or who has been treated previously against bladder transitional cell carcinoma.

8. Method according to claim 1 which comprises the extraction of the sample, either to obtain an extract of proteins or an extract of total RNA.

9. Method according to claim 1 wherein that the detection and quantification of the FGFR3 protein comprises a first step, in which the protein extract of the sample is placed in contact with a composition of one or more specific antibodies, against one or more epitopes of the FGFR3 protein, and a second step, in which the complexes formed by the antibodies and the FGFR3 protein are quantified.

10. Method according to claim 9 wherein said antibodies are monoclonal or polyclonal antibodies, intact or recombinant fragments of antibodies, combibodies and Fab or scFv antibody fragments, specific against the FGFR3 protein; said antibodies being human, humanised or of non-human origin.

11. Method according to anyone of claims 9 or 10 wherein the techniques used in the detection and quantification of the complexes formed by antibodies and the FGFR3 protein are selected from the group comprised by western-blot, ELISA (Enzyme-Linked Immunosorbent assay), RIA (Radioimmunoassay), Competitive EIA (Competitive Enzyme Immunoassay), DAS-ELISA (Double Antibody Sandwich-ELISA), immunocytochemical or immunohistochemical techniques, techniques based on the use of biochips or protein microarrays that include specific antibodies, assays based on the precipitation of colloidal gold in formats such as dipsticks; or by affinity chromatography techniques, ligand binding assays or lectin binding assays.

12. Method according to claim 1 wherein the detection and quantification either of the mRNA of the *FGFR3* gene comprises a first step of amplification of the mRNA that is present in the extract of total RNA, and a second step of quantification of the amplification product from the mRNA of the *FGFR3* gene.

13. Method according to claim 12 wherein the amplification of the mRNA of the *FGFR3* gene comprises converting said mRNA into cDNA by reverse transcription (RT) and amplification thereof by the polymerase chain reaction (PCR).

14. Method according to claim 13 wherein the amplification of the mRNA of the *FGFR3* gene is performed qualitatively or quantitatively, by RT-PCR using primer oligonucleotides, where the sequences of the primers used to amplify the sequence of the *FGFR3* gene are SEQ ID NO. 1 and SEQ ID NO. 2.

15. Method according to claim 1 wherein the detection of the mRNA of the *FGFR3* gene is performed with specific probes either of mRNA or of the corresponding cDNA of the *FGFR3* gene, by techniques such as northern-blot or northern transfer.

16. Method according to claim 1 wherein the detection and quantification of the mRNA of the *FGFR3* gene is performed by Real time quantitative RT-PCR (Q-PCR).

17. An *in vitro* method to identify and evaluate the efficacy of therapeutic compounds against cancer bladder transitional cell carcinoma that comprises:

- a) placing in contact a culture of bladder tumour cells (with uncontrolled proliferation) with the candidate compound, in the appropriate conditions and for a suitable time for these to interact,
- b) detecting and/or quantifying the expression levels of the *FGFR3* gene or the FGFR3 protein, or both, and
- c) comparing said expression levels with those of the control cultures of tumour cells not treated with the candidate compound.

18. An oligonucleotide selected from the group consisting of the oligonucleotide of SEQ ID NO. 1, the oligonucleotide of SEQ ID NO. 2, and mixtures thereof.

19. A kit that comprises the oligonucleotide of SEQ ID NO. 1 and the oligonucleotide of SEQ ID NO. 2.

20. Kit according to claim 19 for detecting detecting the presence of the bladder transitional cell carcinoma in an individual, for determining the stage or severity of this cancer in an individual or for monitoring the effect of the therapy administered to the individual with this cancer.